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Molecular biomarker analysis — Requirements for microarray detection of specific nucleic acid sequences

Analyse moléculaire des biomarqueurs — Exigences relatives à la détection sur microréseaux de séquences d'acides nucléiques spécifiques

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Contents

Page

Foreword ..... **iv**
Introduction ..... **v**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Principle **3**
4.1 DNA microarray platform assay 3
4.2 Microarray design and manufacture 3
4.2.1 General 3
4.2.2 Control probe sequences and targeted probes 3
4.2.3 Analytical power of microarray assay 4
4.3 Validation of hybridization specificity 4
4.3.1 Theoretical assessment of specificity 4
4.3.2 Experimental assessment of specificity 4
4.3.3 Experimental assessment of cross-hybridization 4
4.4 Interlaboratory validation of qualitative methods 5
4.4.1 General 5
4.4.2 Detection limit 5
4.4.3 Probability of detection 6
4.4.4 Limit of detection for microarray platform 6
4.4.5 Range of reliable signal 6
4.4.6 Test sample 6
4.4.7 Measuring system 6
4.4.8 Estimation of measurement uncertainty 7
4.4.9 Microarray reagents 7
5 Expression of results **7**
5.1 General 7
5.2 Expression of a negative result 7
5.3 Expression of a positive result 7
5.4 Expression of inconclusive or ambiguous results 8
6 Test report **8**
Annex A (informative) **Practical determination of limit of detection for microarray platform (LODP)** **9**
Bibliography **13**

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34 *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

This second edition cancels and replaces the first edition (ISO 16578:2013), which has been technically revised.

The main changes are as follows:

— Annex A has been added to provide the practical determination of limit of detection for microarray platform (LODP).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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Introduction

Available methods for nucleic acid sequence discovery using nucleic acid containing samples are based on three technologies: the polymerase chain reaction (PCR), deoxyribonucleic acid (DNA) sequencing and oligonucleotide microarrays.[1]

DNA microarrays are used in biomarker identification and the measurement of gene expression. International harmonization efforts of experimental methods for microarray experiments began with the "Minimum Information about a Microarray Experiment (MIAME)—toward standards for microarray data"[2] and the US Food and Drug Administration's critical path project the Microarray Quality Control project (MAQC),[3][4] which began in 2005 and focused on technical aspects of gene expression measurements, robust technology platforms and the development of accurate and reproducible multivariate gene expression-based prediction models.

DNA microarrays are made either by chemically synthesizing DNA probes on a solid surface or by attaching pre-made DNA probes to a solid surface, e.g. a microplate or coated bead. Microarray assays can be designed to detect multiple single nucleotide polymorphisms (SNPs) simultaneously. High density oligonucleotide microarrays synthesized *in situ* using techniques such as photolithography, ink-jet deposition and robotic arraying of PCR products and pre-synthesized oligonucleotides are manufactured for detecting specific sequences over a wide range of variability.[5] This technology continues to develop along with PCR and next generation sequencing (NGS).

DNA microarrays are typically used to probe a solution of mixed labelled nucleic acids: hybridization of the labelled targets to the fixed probes on the array is detected, and their relative concentration to the remaining nucleic acid species in solution is measured. By generalizing to a very large number of spots of DNA, an array can be used to quantify an arbitrarily large number of different nucleic acid sequences in solution.[6]

Microarray technologies are used in food analysis for detection and identification of genetically modified organisms (GMO) analysis and other biomarkers.[7][8][9][10][11][12] The focus of this document is DNA microarray-based methodologies for food products and products of agriculture.

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Molecular biomarker analysis — Requirements for microarray detection of specific nucleic acid sequences

1 Scope

This document specifies verification and validation parameters and processes for microarray detection and identification of specific nucleic acid sequences.

This document provides recommendations and protocols for:

— microarray design and manufacture;

— validation of hybridization specificity;

— interlaboratory validation of qualitative methods;

— determination of limits of detection for a microarray;

— determination of range of reliable signals;

— criteria for assessing technical performance of the microarray platform:

This document is applicable to all methods that use microarrays for detection of nucleic acids.

It does not apply to the following protocols:

— quantitative measurement;

— requirements for sample preparation prior to DNA microarray experiments.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TS 16393, *Molecular biomarker analysis — Determination of the performance characteristics of qualitative measurement methods and validation of methods*

ISO 16577, *Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

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3.1
limit of detection for microarray platform
LODP
lowest relative quantity of the *external measurement standard* (3.11) (or reference material) for which a positive identification can be achieved with reasonable or previously determined confidence or both in a defined matrix using a specific analytical method

Note 1 to entry: In qualitative testing, an estimate of the LOD is measured at the chosen probability of detection (POD).

3.2
range of reliable signal
concentrations of target sequence for which a method can provide results where the output signal is proportional to the concentration and/or copy number of the *external measurement standard* (3.11) (or reference material)

Note 1 to entry: Direct of derived proportionality is acceptable in this range.

3.3
DNA microarray
DNA chip
solid substrate where a collection of *probe DNA* (3.6) arranged in a specific design is attached in a high-density fashion, directly or indirectly, that assays large amounts of biological material using high-throughput screening methods

3.4
analytical power
power
probability that an analyte will not go undetected if it is present

3.5
sensitivity
smallest treatment response that will be detectable

3.6
probe DNA
single-strand nucleic acid defined by its property to target specific nucleic acid sequence by base complementarities, where the stringency of the binding is linked with the length and nucleic acid composition of the probes, along with reaction parameters

3.7
platform
device that supports a *DNA microarray* (3.3) technology

3.8
fluorescence detection
method of detecting hybridization using immobilized *probe DNA* (3.6) by measuring a fluorescence signal

3.9
colorimetric detection
method of detecting hybridization using immobilized *probe DNA* (3.6) by measuring a colorimetric signal

3.10
electrochemical detection
method of detecting hybridization by measuring electric currents of an electrode onto which *probe DNA* (3.6) are immobilized

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3.11
external measurement standard
material or substrate prepared for testing the compatibility of the microarray-based methods of analysis, whose property value is derived as a consensus value based on collaborative experimental work under the auspices of a scientific or engineering group

3.12
cross-hybridization
non-specificity binding of *probe DNA* (3.6) to non-targeted nucleic acid

4 Principle

4.1 DNA microarray platform assay

A microarray platform assay consists at a minimum of the following steps:

— denaturation of the double- or single-stranded DNA or ribonucleic acid (RNA) analyte (DNA sample);

— hybridization of the target(s) to probe DNAs bound to a solid substrate;

— detection of each hybridized target(s) by an electrochemical, colorimetric or fluorescence signal;

— data analysis.

The laboratory shall verify the procedure used for each microarray assay step, using known measurement standards (or reference material) and appropriate controls. Requirements governing verification of DNA microarray-based methods shall be documented.

4.2 Microarray design and manufacture

4.2.1 General

The DNA microarray and device for analysis shall be validated as an integrated measurement system for specific nucleic acid analysis. The performance of a DNA microarray shall be specified in combination with its analytical device. The DNA microarray and the device that analyses it should specify the performance in an integrated state and evaluate its reliability.[13]

4.2.2 Control probe sequences and targeted probes

A DNA microarray analysis shall have the following types of probe DNAs incorporated:

— external measurement standards (or reference material);

— a positive control;

— a negative control;

— the nucleic acid sequence of interest.

It shall be designed to be verifiable.

The immobilized DNA probes for signal quality control, including but not limited to the positive and negative controls, shall be included in the microarray design as replicates located in different positions on the microarray.[14] Probe DNA design shall consider the Tm value, GC ratio and sequence specificity of the nucleic acid oligonucleotide. Oligonucleotide probe sequence information shall be provided according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature code for nucleic acids.[15] In order to improve legibility between “G” and “C”, lower-case “g” should be used in the description (i.e. C, g, A, and T shall be used to indicate bases). The quality of probe DNA shall be ensured by an appropriate method, e.g. spectroscopic analysis, mass-spectroscopy analysis.

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